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Single-Pair SAXS for Analyzing a Conformational Ensemble of Heavy Atom-Labeled Small Molecules

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1 Introduction

It is difficult to obtain unbiased spatial information on the dynamic conformational ensemble of a molecule in solution. There are several experimental methods, but they have inherent problems in achieving this demanding task. One promising approach is small-angle X-ray scattering (SAXS) by molecules in a liquid solution. A scattering curve, I(q), obtained from a SAXS experiment can be converted to a pair distribution function, P(r). The function P(r) describes the distribution of distances between all pairs of atoms in a molecule (Fig. 1, *upper panel*). If we introduce a pair of strong scatterers as probes into a molecule and selectively measure the SAXS profiles from the two scatterers, then the P(r) function will describe the spatial distribution of the inter-probe distance (Fig. 1, *lower panel*). The background SAXS profile originating from the other atoms in the molecule must be suppressed. Gold nanocrystals have been successfully used as strong scatterers[1]. Gold nanocrystals are attached *via* a gold-thiol linkage to the terminal and middle positions of double-stranded (ds) DNA fragments, ranging from 10 to 35 bp. The background scattering from the DNA part is suppressed by the combination with scattering data from singly labeled and unlabeled DNA samples, according to the equation $I_2(q) - I_1(q) - I_1(q) + I_0(q)$. Note that there are two different scattering data corresponding to two singly labeled species. The typical distance ranges are limited between 50 and 150 Å in the gold-labeled DNA experiments. The large size (diameter of 12-14 Å) and the size distribution of the gold nanocrystals, and the necessity of the linker for immobilization, reduce the usefulness for monitoring the dynamics within a *small* molecule. Heavy single atoms directly attached to a target molecule without a linker would be preferable in this respect but have not been experimentally assessed so far.

2 Experiment

Small-angle X-ray scattering was measured at the beamline BL6A of the Photon Factory. The sample volume was 40 μL, and a microflow cell was used to reduce X-ray irradiation damage. The X-ray wavelength was set at 1.5 Å and the sample-detector distance was 1.0 m. Data were collected on a PILATUS 1M detector in twenty 20-second exposures per sample. Three scattering profiles were collected from samples of unlabeled, singly labeled, and doubly labeled PEGs dissolved in DMSO at a final concentration of 29 mM. The profiles were added and subtracted according to the equation $I_2(q) - 2 * I_1(q) + I_0(q)$, to extract the X-ray scattering interference pattern between two iodine atoms connected by a flexible PEG chain (Fig. 2).

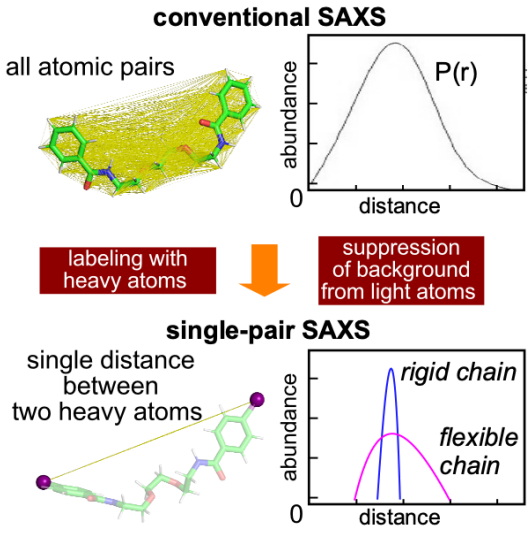

Fig. 1. TOC summary of the single-pair SAXS measurement

We used a modified version of the MATLAB program used in the study on the gold-nanocrystal labeled DNA fragments[1]. The program is available in the supplementary information of ref 2.

3 Results and Discussion

We present a feasibility demonstration to show that the distance distribution between two heavy atoms in small organic molecules in the range of 10 - 30 Å can be experimentally obtained[2]. Iodine was chosen as a strong X-ray scatterer and covalently attached to the ends of a flexible polyethylene glycol [PEG, -(CH_2-CH_2-O-)$_n$]chain (Fig. 2). Since the scattering power of a single iodine atom is much smaller than that of a gold nanoparticle (which consists of 78 gold atoms), the feasibility must be examined experimentally. We refer to the compounds in Fig. 2 as I_2-PEGn, I_1-PEGn, and I_0-PEGn, where I_2, I_1, and I_0

denote the number of iodine atoms, and *n* denotes the number of ethylene glycol units (n = 3, 5, 9). Note that I-PEG*n*-H and H-PEG*n*-I are identical due to the C2 symmetry, and referred to simply as I_1-PEG*n*.

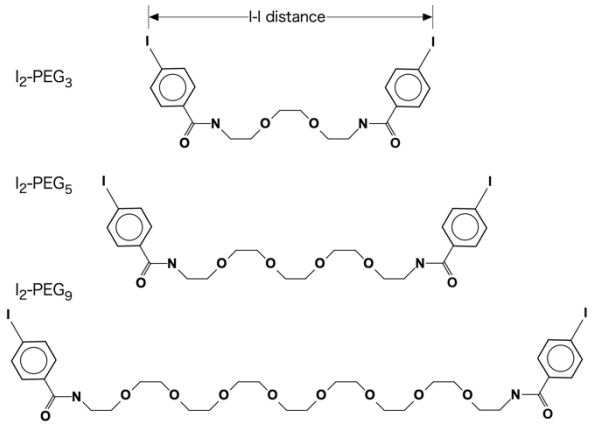

Fig. 2. Chemical structures of the iodine-labeled PEGs. The maximum iodine-iodine (I-I) distances are 25.1 Å for I_2-PEG_3, 31.6 Å for I_2-PEG_5, and 45.2 Å for I_2-PEG.

The distance distribution between the two iodine atoms was obtained by decomposing the interference pattern into a linear combination of interference basis functions between two iodine atoms, positioned at various distances from 0 to 200 Å with 1 Å intervals (Fig. 3). The scattering data in the range of 0.008 Å^{-1} < S < 0.064 Å^{-1} were used for input. The decomposition was performed by the maximum entropy procedure coded in MATLAB.

Our proof-of-concept SAXS experiment was proved useful in extracting distance distributions in the range of 10 – 30 Å. The present single-pair (of heavy atoms) SAXS experiments widen the applicability of SAXS interferometry from ds DNA fragments to flexible small organic compounds in the physical and chemical fields. We also expect to obtain new insights into the spatial dynamics of folded and IDP (intrinsically disordered polypeptide)-state proteins in aqueous solutions for biological applications.

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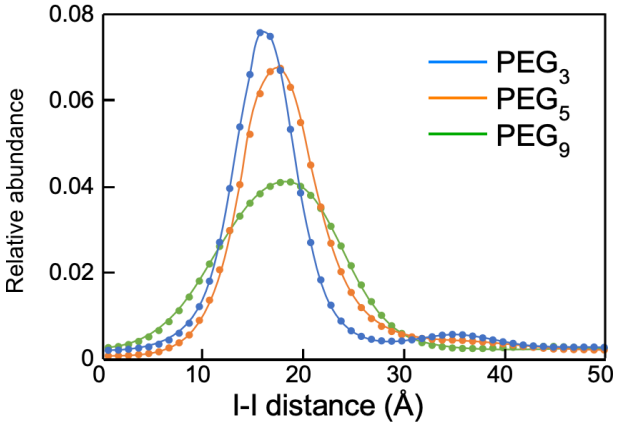

Fig. 3 SAXS derived I-I distance distributions of the double-iodine labeled PEG chains.

References

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